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ENVIRONMENT FRIENDLY ANTIBACTERIAL AND UV PROTECTIVE FINISH ON COTTON USING SYZYGIUM CUMINI (L.) LEAVES EXTRACT

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ABSTRACT

The present study was conducted to develop antibacterial and UV protective cotton fabric by using plant extract. Syzygium cumini (L.) leaves extract was extracted through soxhlet method and was applied on cotton fabric by using pad dry cure process. Phytochemical analysis of S. cumini (L.) leaves extract indicated presence of tannin, flavonoids, saponin and phenols and exhibited antibacterial activity against gram-positive bacteria with zone of inhibition of (6.0 – 11.16 mm for B. subtilis and 4.83 -10.0 mm for S. aureus) and sun protective property with 23.91 – 25.01 SPF value at different concentrations. Cotton fabric finished with S. cumini (L.) leaves extract exhibited improvement in bacterial resistance with per cent reduction in the bacterial count of the finished fabric by 95.67% for S. aureus and 94.70% for B. subtilis as well as exhibited high UPF value (48.1), providing excellent protection when compared to untreated control fabric. It can be concluded that the S. cumini (L.) leaves extract contains medicinal potential and not only provides protection from environmental hazard but also safeguards the environment, prevents pollution and promotes eco-friendly textiles.

KEYWORDS: Antibacterial, Cotton Fabric, Syzygium cumini (L.) & Ultraviolet Protection Factor

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Original Article

INTRODUCTION

Adaptation of clothing for covering our bodies pre-dates to the historic age. Clothes have fulfilled a variety of functions, ranging from primarily awarding protection, warmth as well as being a symbol of fashion statement. In recent years, due to aggravation in health related issues, the collaboration between medical personnel and textile chemistry technologies has led to the evolution of innovative medico-functional applications for textiles. Such applications include the addition of pharmaceutical herbal ingredients into the clothing, which then protect the skin and human body from environmental hazards such as UV rays and microbes. Cotton being a natural fiber offers an ideal environment for microbial growth (Salah, 2011) because it retains oxygen, water and nutrients as well as reported to provide least UV protection[8]. Many chemical substances such as viz., triclosan, quarternay ammonium compounds which acts as antimicrobial agents and UV protective agents viz., titanium dioxide and ceramic materials are available for textile finishing (Hussain and Jahan, 2010; Thilagavathi and Kannaian, 2010). However, due to their synthetic nature, natural products in textile finishing are gaining significant momentum (Lee *et al*., 2009). Use of plant extracts not only provides protection from environmental hazard but also safeguards the environment, prevents pollution and promotes eco-friendly textiles. Use of such products also ensures the health benefits to the individual as well as the masses. Such textiles prevent pollution by avoiding the

use of lethal chemicals which are used in finishing. It also encourages tree plantation because the herb and plant parts used are the main source of raw material. Together it provides manure to the soil, plants as well as the waste generated by eco-friendly finishing would serve the same purpose.

Natural products are a source of new chemical diversity and are the choice of today's world. The sources of natural product are plants, animals and microorganisms. Among them, plants and plant products are more reliable for its renewability and therefore considered as catalyst for human welfare. They are the primarily required materials for health care system in some parts of the world (Mahesh *et al.,* 2011). Plants have their own self defense mechanism and protect themselves from UV rays and microbes due to the presence of substances known as phytochemicals (Phyto-from greek- *phyto* meaning 'plant') (Yalavarthi and Thiruvengadarajan, 2013). These phytochemicals are divided into primary and secondary metabolite. Primary metabolites are the compounds involved in the metabolic pathway, which are common to all living organisms (Dewick, 2009). On the other hand, secondary metabolites function as defense and signal compounds that are necessary for the plant's survival and reproductive ability (Wink, 2003). Secondary metabolites extracted from plants such as phenols, flavonoids and anthraquinone have been considered as sunscreen agents because of their ultraviolet absorption property (Ramu *et al*., 2012). The effect of various plant extracts such as tulsi (leaf), pomegranate (rind), neem (leaves) on bacteria and fungi has been reported (Sathianarayanan *et al*., 2010; Hooda *et al*., 2013). As well, protection from UV radiation can be obtained by plant extracts such as *Q. infectoria* (Gupta, 2005). Although, many plants rich in antibacterial and UV protective agents are reported but the work on the exploration of *Syzygium cumini* (L.) leaves extract and its application on textiles is not yet documented.

S. cumini (L.) (Myrtaceae) is commonly known as jamun, jaman, dahut in Hindi and black plum tree, Indian blackberry, Malabar plum in English. It is a large, evergreen widely distributed forest tree of India, Sri Lanka, Malaysia and Australia. The entire plant is used for traditional medicinal purposes which include diabetes, curing diarrhea, dysentery, obesity etc. Leaves and bark have been reported as the most powerful parts of the tree as traditional medicine, where leaves posses antibacterial properties and are used to strengthen teeth and gums, taken orally (as tea) to treat diabetes in Brazil and have been reported as antihyperglycemic, antinflamation, antioxidant effects (Chaudhary and Mukhopadhay, 2012; Sharma *et al*., 2012). Based on the review, the present study was undertaken to study medicinal potential of leaves extract of *S. cumini* (L.) with following objectives:

- Analysis of *S.* cumini (L.) leaves extract in regard to phytochemical, antibacterial and sun protective properties.
- Assessment of treatments efficacy with respect to ultraviolet protection and antibacterial properties of cotton fabric.

MATERIALS AND METHODS

Materials

To develop antibacterial and UV protective textile, various chemicals/auxiliaries such as methanol (solvent), Americos Amylase 543 (desizing agent), Palkascour (scouring agent), Nutrient agar, Brain heart infusion broth, Fixa prêt F-Eco (resin cross-linking agent), magnesium chloride (catalyst), 100% cotton (plain weave) fabric was procured from respective industries.

Preperation of *S. Cumini* (L.) Leaves Extract

Fresh, mature leaves of *S. cumini* (L.) (jamun) was collected from CCS, Haryana Agricultural University, Hisar, during the month of April 2014. The collected plant material was thoroughly washed under tap water, rinsed in distilled water, shade dried and was grounded in powder form by using an electric grinder. Hot methanol extract was prepared by using soxhlet extraction process. About 40 g powder was extracted with 250 ml of methanol at 55-60^{0}C for 4 hours. The solvent containing active constituents was transferred to rota vapour to evaporate the solvent and to get solid extract. The extract was kept in refrigerator at 4 ^{0}C, to be used for further study (Choudhury *et al*., 2012).

Qualitative Phytochemical Screening

Freshly prepared methanol leaves extract of *S. cumini* (L.) was subjected to phytochemical analysis to find the presence of the following secondary metabolites such as flavonoids, alkaloids, tannins, saponin, phenols by using the methods described by Tiwari *et al*. (2011), Gopinath *et al.* (2012), Vermani *et al.* (2013) and Saidulu *et al.* (2014).

Pre-Treatment of Cotton Fabric

To ensure complete wetting and uniform absorbency of the extract and other finishing agents during finishing process, cotton fabric was subjected to different pre-treatments.

Enzymatic Desizing

The greige woven cotton fabric was treated with 3% concentration of alpha amylase (Americos Amylase 543), 1:20 material to liquor ratio at 60^{0}C for 45 min reaction time at pH 6-7 level. The fabric was thoroughly rinsed with hot water followed by cold water and then dried at 80^{0}C using hot air oven (Vigneswaran *et al*., 2013).

Enzymatic Scouring

The desized woven cotton fabric was treated with 2% pectinase (Palkascour) concentration with 1:20 material to liquor ratio for 45 min duration at 40ºC at pH 8. After the treatment, the fabric was thoroughly rinsed with hot water followed by cold water and then dried at 80^{0}C using hot air oven (Ragendran *et al*., 2011).

Analysis of Sun Protection Factor of *S. Cumini* (L.) Leaves Extract

Five and 10 mg of extract was weighted accurately and dissolved in 1 ml of solvent to prepare 5 and 10 mg/ml solution. The prepared solution was subjected to the evaluation of sun protection factor by using Double Bean UV-Vis Spectrophotometer. Formulae given by Mansur *et al.* (1986) and values given by Sayre *et al.* (1979) were utilized to calculate sun protection factor value by using following equation :

$$SPF = CF \times \sum_{290}^{320} EE \times I \times Abs$$

Where, EE (λ) – Erythemal effect spectrum, I (λ)–Solar intensity spectrum, Abs–Absorbance of sunscreen product and CF–Correction factor (=10). The values of EE x I were constant and predetermined as shown in table 1.

Table 1: Values of EE x I used in the Calculation of SPF

Wavelength (λ nm)	EE x I (Normalized)
290	0.0150
295	0.817
300	0.2874
305	0.3278

Table 1: Contd.,	
310	0.1864
315	0.0839
320	0.0180
Total	**1**

EE–Erythermal effect spectrum and I–Solar intensity.

Qualitative Analysis of Antibacterial Property of *S. cumini* (L.) Leaves Extract

Agar well diffusion method was employed to study the antibacterial property of plant extracts. For qualitative analysis, different concentrations i.e. 5, 10, 20, 30 mg/ml of *S. cumini* (L.) leaves extract was studied, to analyse the effect of concentration of *S. cumini* (L.) leaves extract on bacteria. The experiments were conducted under controlled conditions in the Laminar Flow Chamber.

Test Microorganism

The pure cultures of two common human pathogenic bacteria (Gram-positive) i.e. *Staphylococcus aureus* and *Bacillus subtilis* were obtained from the Department of Veterinary Microbiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and sub-cultured in nutrient agar plate. At least three to five isolated colonies of same morphological characters from the agar plates of each microorganism were selected and the culture was aseptically transferred into the tube containing 5 ml of brain heart infusion broth. The inoculated broth tubes were incubated at 37^0C for 24 h. The growth in the tubes was observed in the form of turbidity and tubes were stored at 4^0C for further work.

Agar Well Diffusion Method

The modified agar well diffusion method of Dey *et al.* (2010) was employed to study the antibacterial activity of *S. cumini* (L.) leaves extract. The nutrient agar medium (28 g nutrient agar in 1000 ml distilled water) was prepared. After autoclaving, the media was poured into sterilized petri plates with a uniform thickness of approximately 4 mm. The agar plates were allowed to solidify at ambient temperature and were used after 24 h. Five uniform wells were prepared with the help of sterile cork borer of 5 mm diameter in agar plates. The entire agar surface was then inoculated by sterile cotton swab dipped in bacterial culture and left for 3 to 4 min. Hot methanol extract of *S. cumini* (L.) in different concentrations were added to the grooves with one control. The plates were incubated for 24 h at 37^0C. Plates were examined and zone of inhibition of the bacteria growth was measured in mm by using diameter measurement scale. All the assays were performed in triplicate and expressed as average values.

Application of Finish

Application of finish on cotton fabric was performed by using pad-dry-cure process. The fabric was immersed in the finishing solution (containing 11% *S. cumini* (L.) leaves extract, 1:20 material to liquor ratio, 60 g/l resin cross linking agent, 10 g/l magnesium chloride) at 5.5 pH for 30 minutes. The fabric was then passed between the rollers at 2 kg/cm^2 pressure, to squeeze out excess liquid from the fabric. The fabric was then dried at 110^0 C for 5 min and cured at 150^0 C for 3 min in a curing chamber.

Quantitative Antibacterial Test Method

Antibacterial property of control (unfinished) and finished fabrics against gram positive bacteria i.e., *S. aureus* and *B. subtilis* was quantitatively determined by using AATCC Test Method-100. Fifty ml brain heart infusion broth was prepared in 100 ml conical flasks. These flasks were inoculated with 100 micro liter of different bacterial cultures i.e.

S. aureus and *B. subtilis*. The fabric samples were aseptically transferred in the above described conical flasks. These flasks were incubated at 37^0C for 24 h in a shaker incubator at 121 rpm. The flasks were removed from the incubator and serial dilutions were made up to 10^6 for *S. aureus* and 10^7 for *B. subtilis.* Hundred micro liter of diluted culture from each tube was taken aseptically and spread on nutrient medium plates under laminar air flow chamber. The plates were incubated in inverted position at 37^0 C for 24 h. The total colony forming units were calculated using following formula:

Colony forming units = No. of colonies x Dilution factor x Dilution

= CFU/ml

The results were enumerated as percentage reduction in the bacterial count of the finished fabric in comparison to the bacterial count of control fabric and was calculated as under:

$$\text{Reduction in bacterial count (\%)} = \frac{\frac{\text{CFU}}{\text{ml}}\text{of control fabric} - \frac{\text{CFU}}{\text{ml}}\text{of finished fabric}}{\frac{\text{CFU}}{\text{ml}}\text{of control fabric}} \text{ x } 100$$

Assessment of Ultraviolet Protection Factor (UPF)

To determine the ultraviolet radiation blocked or transmitted by cotton fabric intended to be used for UV protection, UVR TRANSMISSION AATCC-183:2004 test method was used. The transmission of ultraviolet radiation (UV-R) was determined in the wavelength range of 280-400nm by using Compsec M 350 UV-Visible Spectrophotometer. Ultraviolet protection factor (UPF) was calculated using mean percentage transmission in UVA region (320-400 nm) UVB region (280 -320 nm) according to the following equation:

$$UPF = \frac{\sum_{\lambda\,290}^{400} E\lambda \; x \; S\lambda \; x \Delta\lambda}{\sum_{\lambda\,290}^{400} E\lambda \; x \; S\lambda \; x \; T\lambda \; x \Delta\lambda}$$

Where: Eλ = relative erythermal spectral effectiveness, Sλ= solar spectral irradiance, Tλ= average spectral transmission of the specimen, ∆λ= measured wavelength interval (nm)

RESULTS AND DISCUSSIONS

Qualitative Phytochemical Screening

Qualitative phytochemical screening of methanol leaves extract of *S. cumini* (L.) indicated presence of tannin, flavonoids, saponin, phenols whereas alkaloids was found to be absent as shown in table 2. The results of phytochemical analysis of *S. cumini* (L.) are in agreement with the work of Gopinath *et al.* (2012) who reported the presence of same phytochemicals in *S. cumini* (L.) leaves extract.

Table 2: Qualitative Phytochemical Screening of *S. Cumini* (L.) Methanol Leaves Extract

Phytochemicals	*S. Cumini* (L.)
Alkaloids	-
Tannin	+
Flavonoids	+
Saponin	+
Phenols	+

+ indicates presence and – indicates absence.

Antibacterial Activity

The *S. cumini* (L.) leaves extract exhibited antibacterial activity against gram positive bacteria (*S. aureus* and *B. subtilis*), which increased with increase in concentration of extract. The zone of inhibition at different concentration of extract (5, 10, 20, 30 mg/ml) as shown in table 3 was in the range (6 – 11.16 mm) for *B. subtilis* and (4.83 – 10.0 mm) for *S. aureus*, with maximum zone of inhibition at 30 mg/ml concentration (30 mg/ml) (plate 1).

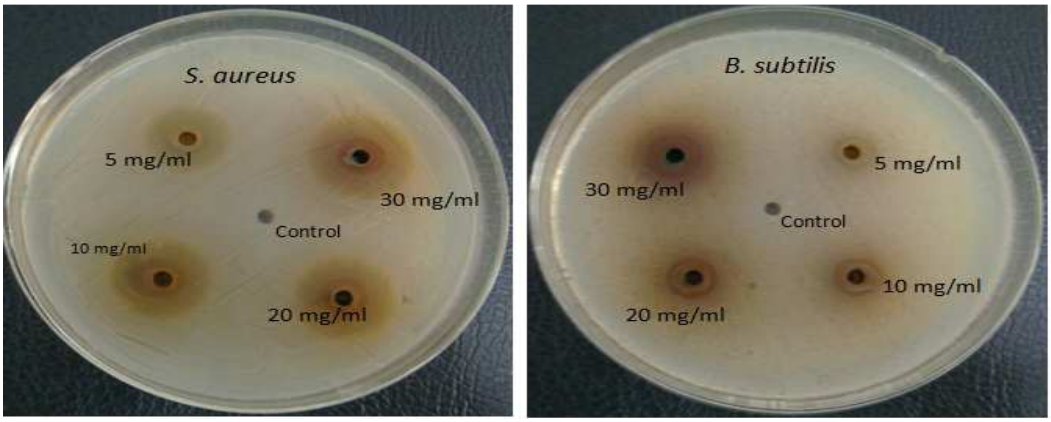

Plate 1: Zone of Inhibition against Gram-Positive Bacteria

Table 3: Screening of Antibacterial Activity of *S. cumini* (L.) Leaves Extract

Concentration (mg/ml)	Zone of Inhibition (mm)	
	B. subtilis	***S. aureus***
5	6.0	4.83
10	9.0	8.0
20	10.16	9.83
30	11.16	10.0
Control	0.0	0.0

UV Absorption and Sun Protection Factor

The ability of plants to defend themselves from UV radiation makes them a good source of interest and draws attention of researchers towards their sun protective capabilities. The UV absorption activity and sun protection factor (SPF) of *S. cumini* (L.) leaves extract was studied at 5 and 10 mg/ml concentration. Results showed that at Ultraviolet range i.e. 290-320 nm, *S. cumini* (L.) has ability to absorb ultraviolet radiation, which increased with increase in concentration of extract as shown in table 4.

Table 4: UV Absorption and Sun Protection Factor of *S. cumini* (L.)

Wavelength	Absorbance (λ)	
	5mg/ml	**10 mg/ml**
290	2.509	2.699
295	2.481	2.636
300	2.391	2.526
305	2.404	2.49
310	2.365	2.431
315	2.321	2.48
320	2.287	2.383
SPF Value	**23.91**	**25.01**

Assessment of Antibacterial and UV Protective Properties of Finished Fabrics

The antibacterial and UV protective properties of finished fabric was compared to control (unfinished) fabric by calculating the per cent reduction in bacterial count as well as by studying the change in UV protection category of fabric. The data presented in Table 5 reveal that the bacterial count of control fabric was $32.4x10^7$ for *S. aureus* and $37.8x10^8$ for *B. subtilis*. Whereas after the application of finish, the bacterial count observed was $1.4x10^7$ for *S. aureus* and $2.0x10^8$ for *B. subtilis*, exhibiting per cent reduction in the bacterial count of the finished fabric by 95.67% for *S. aureus* and 94.70% for *B. subtilis* when compared to the control fabric, as evident from plate 2.

Table 5: Antibacterial Property of Finished Fabrics

Properties / Cotton Fabrics	Antibacterial			
	***S. aureus* CFU/ml**	**% Reduction**	***B. Subtilis* CFU/ml**	**% Reduction**
Control	$32.4x10^7$	-	$37.8x10^8$	-
Finished	$1.4x10^7$	95.67	$2.0x10^8$	94.70

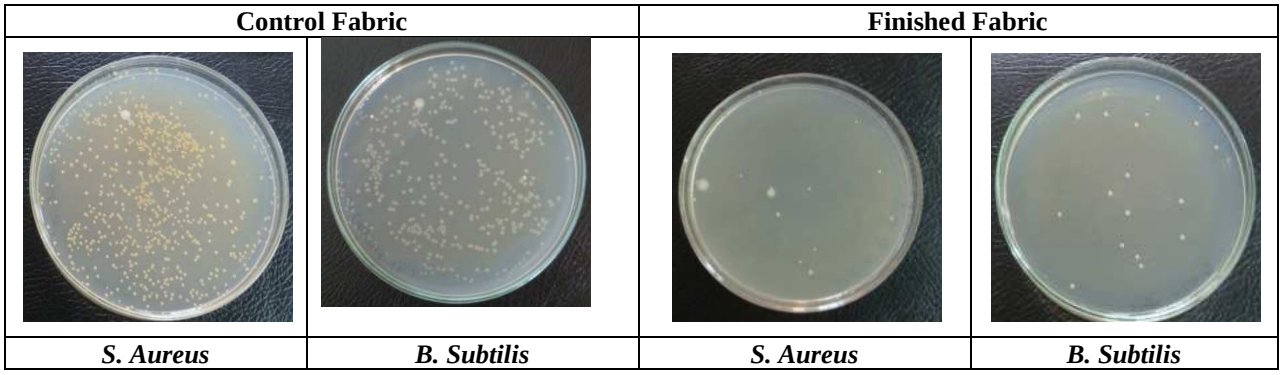

Plate 2: Bacterial Growth on Control and Finished Fabrics

The mean UVA per cent transmission of control (unfinished) cotton fabric was 7.70 whereas mean UVB per cent transmission observed was 8.57 and the mean UPF value was 10.9, providing no protection. After the finishing treatment, decrease in UVA (2.64) and UVB (2.08) per cent transmission was observed and UPF value of cotton fabric after application of finish was observed as 48.1, providing excellent protection as shown in Table 6.

Table 6: UV Protective Property of Finished Fabrics

Properties / Cotton Fabrics	Ultraviolet Protection				
	Mean			UPF	
	UVA%	**UVB%**	**UPF value**	**Range**	**Protection Category**
Control	7.70	8.57	10.9	-	No protection
Finished	2.64	2.08	48.1	40-50	Excellent

DISCUSSIONS

Textile materials made up of natural fibers such as cotton are susceptible to microbial attack (Bajpai *et al*., 2011). This is evident from the results presented in Table 3, which reveals high bacteria count on untreated cotton fabric. The application of *S. cumini* (L.) leaves extract on cotton fabric exhibited 95.67% and 94.70% percent reduction in bacterial count which may be attributed to the active antibacterial compounds such as tannin, saponin, flavonoids and

phenols in *S. cumini* (L.) leaves extract, as revealed by the phytochemical analysis performed during this study. The antibacterial activity of such phytochemcials was reported by earlier studies and revealed that they inhibites the growth of microbes in many ways such as by inhibiting protein synthesis, inferring with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathway, inferring with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms (Sumitra and Kaneria, 2011). Also, the qualitative antibacterial analysis clearly reveals the potential of *S. cumini* (L.) leaves extract against *S. aureus* and *B. subtilis* which increased with increase in concentration of extract as shown in plate 1. This explains, plants containing natural ingredients have potential to reduce bacterial growth and as reported these herbal extracts are slow but effective and are safe to human being as well as environment. The results are supported by the findings of Gupta and Laha (2007) who reported the good antibacterial activity of cotton fabric when treated with the tannin-rich extract of *Quercus infectoria* (QI) plant in combination with alum and copper against *B. subtilis.*

Studies reveal that the sun-blocking properties of a textile are enhanced when a dye, pigment, delustrant, or ultraviolet absorber finish is present that absorbs ultraviolet radiation and blocks its transmission through a fabric to the skin (Hustvedt and Crews, 2005). *S. cumini* (L.) leaves extract was also evaluated for sun protective and UV protective properties in the present study.

Application of *S. cumini* (L.) leaves extract rendered excellent UV protective property to cotton fabric as is evident from table 6. The result explains that *S. cumini* (L.) leaves extract possess sun protective properties which may be rendered to the presence of flavonoid such as quercetin present in *S. cumini* (L.) leaves as reported provides protection against UVA and UVB radiations[5]. The results are in line with the study conducted by Maske *et al.* (2012) who revealed that the ethanolic extract of *S. cumini* (L.) had UV absorbing ability and rendered the activity to the presence of flavonoids in the extract, as flavonoids absorb light and help to protect photosensitive substances in the flowers and leaves. Good to high sun protective activity against UV radiation of *Zingiber officinale and Mentha* leaves is reported by Suva (2014) and Gupta (2013). A study conducted by Hustvedt and Crews (2005) found that the naturally coloured cotton i.e. in brown, green and tan, exhibited significantly higher UPF values than conventional cotton and this property can be due to the tannin and phenolics naturally presented as vaculoles in the fibre lumen of naturally coloured cotton (Kranthi, 2014). Thus, high UV protective property of finished fabric can be attributed to the presence of tannin, flavonoids, phenols in *S. cumini* (L.) leaves extract as reported, plant-derived phenylpropanoids (especially flavonoids) are of great medicinal interest, especially as free radical scavengers, UV screens and UV absorbers (Katerova *et al*., 2013).

CONCLUSIONS

Thus, from the findings, it can be concluded that application of UV protective and antibacterial finish on cotton fabrics by using *S. cumini* (L.) leaves extract improved UV protective and antibacterial properties to a greater extent, leading to excellent protection. This new plant source exhibiting, antibacterial and UV protective property can be used for development of medical textiles as well as for apparels for daily use. This study also provides a new source for natural plant material which can be combined with new technologies such as microencapsulation and nanotechnology to develop effective and durable textile materials.

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